Author:
Jane Hurst
Department of Veterinary Clinical Science
University of Liverpool

Correspondence to:
Jane Hurst
jane.hurst@liv.ac.uk
www.liv.ac.uk/abg

National Centre for the Replacement, Refinement and Reduction of Animals in Research

Making sense of scents: reducing aggression and uncontrolled variation in laboratory mice

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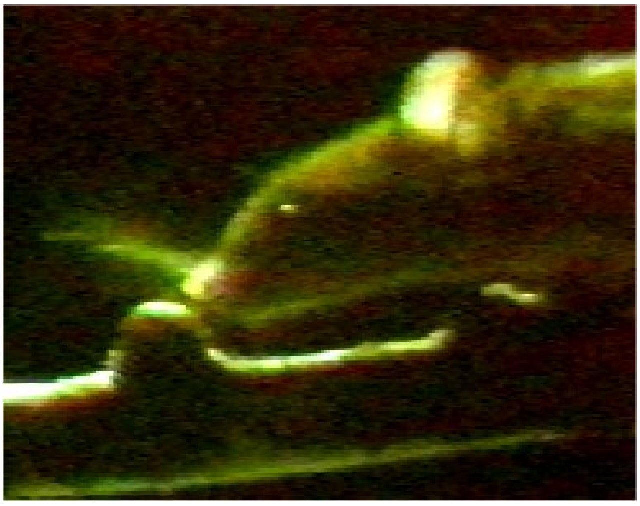
Abstract

Scents are the primary means of communication in mice. They underlie most aspects of their social behaviour and are particularly important in mediating aggressive interactions and status differentiation among males. This competitive aggression can be a major welfare concern and source of uncontrolled variation among laboratory mice. Scents also play an invisible role in priming reproductive physiology and development with additional consequences for immunocompetence, introducing another potential source of uncontrolled variation that could influence many types of experiment. Here, I provide a brief explanation of how wild mice use scents to recognise each other and to control competitive interactions. I then discuss the consequences of this for aggression among laboratory mice and recommend ways to minimize problems through management practices. Known reproductive priming effects are also summarised to show how exposure to scents and cage group size can influence sex hormone levels, reproductive cycling and development. Careful consideration of husbandry and experimental design can also reduce this source of variability.

Keywords: laboratory mice, scent communication, olfaction, aggression, reproductive priming effects, physiology, husbandry, variability, inbred, outbred.



The sensory world of mice is quite different from ours. Watch mice for a few minutes and you soon realise that they are largely led by their noses. Their primary means of communication is through their characteristic, pungent scents. Because humans gain information primarily through vision and hearing, we have little understanding of the information that other animals gain through scents. As mouse scents mediate a wide range of behavioural responses including competitive aggression among males, as well as influencing physiology through priming effects, it is important to understand and control these invisible signals as part of good husbandry and experimental design.

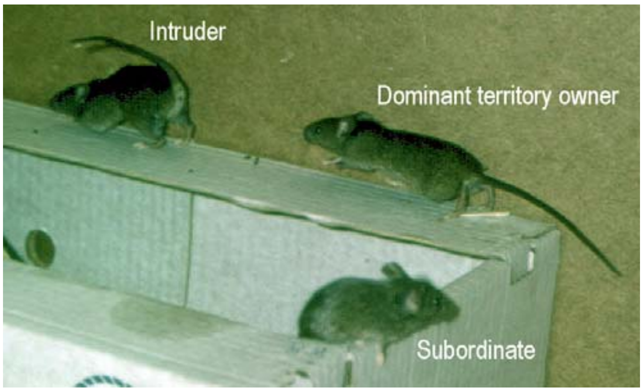
Laboratory mice are derived from wild house mice (*Mus domesticus*, also known as *Mus musculus domesticus*), one of the most flexible and opportunistic of mammal species. Understanding the natural behaviour and ecology of these wild ancestors can provide considerable insight into the requirements of mice in the laboratory.

However, laboratory mice are not wild animals and do not behave 'just like wild mice'. They have been selected for particular characteristics in the laboratory, notably for their ease of handling and breeding and for reduced genetic variation. Many strains have been specifically inbred over many generations to provide animals with fixed genetic characteristics that are virtually genetically identical to each other, a situation which is completely at odds with outbred wild mice and changes their ability to recognise one another. Further, the captive environment and social experience of laboratory animals differs considerably from that in the wild and such experience plays a major role in shaping animal behaviour. In addition to understanding the original adaptations of their wild ancestors, we need to understand the impact of genetic selection, captive housing and restricted social experience on the responses of laboratory animals to work out appropriate captive conditions that maximise welfare in the laboratory and minimise uncontrolled variation.

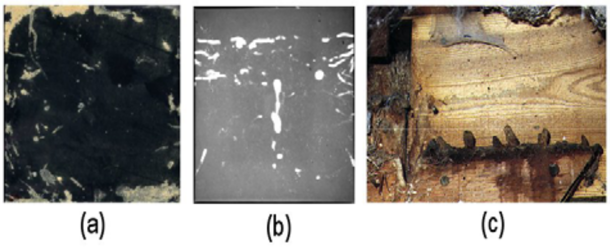
Competitive scent communication among wild house mice

Although we usually think of the natural environments for rodents as grassland, woodland and hedgerows, the preferred habitats of house mice are farms, grain stores, domestic and catering premises where food is concentrated and readily available and buildings provide protection from harsh weather and predators.

Under these circumstances, mice do not need to compete for food and populations can build up to very high densities due to rapid reproduction and limited dispersal from highly favourable habitats. Instead, mice compete for breeding opportunities. Mice live in territorial social groups, each with a single dominant male territory owner while females may range over several neighbour male territories. Dominant territory owners are relatively tolerant of familiar subordinates that live within the territory, attacking them to maintain status differentiation but with a low level of aggression as long as subordinates avoid competing for dominance. By contrast, territory owners are highly intolerant of intruders or resident males that try to compete and these males are driven from the territory through persistent chasing and injurious bites. Scent cues on the body of each animal and deposited in the environment are essential in maintaining this social system, allowing animals to identify each individual, their status and where they reside.



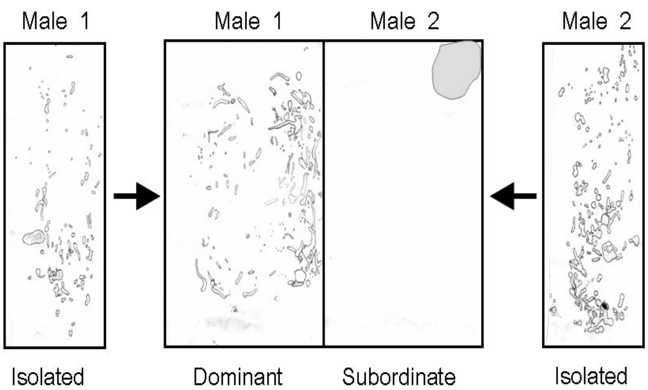
Although there are specialised scent glands on the body (particularly around the face), urine is a main source of scents in mice and is used to liberally scent mark their home area (faeces are not used for scent communication in this species). Mouse urine contains a complex mixture of small volatile odorants that are deliberately excreted in urine as chemical signals (pheromones). Some of these are bound to a high concentration of non-volatile proteins termed major urinary proteins or MUPs. The combination of volatile and non-volatile pheromone components provides information on both the identity and current status of the urine owner (see Table 1). Urine is not simply evacuated but deposited as scent marks in many small streaks and spots on the substrate throughout their home area. Although all residents add to these scent marks to advertise their membership of the resident group, the dominant territory owner marks at a much higher rate.



Sticky urine scent marks can be seen (a) on smooth non-absorbent surfaces such as this acetate sheet, or (b) when visualised on absorbent paper using ultra-violet light. (c) In the wild, some sites may be marked repeatedly so that communication 'posts' of urine and dust can build up like small stalagmites.

Like most territorial mammals, mice use scent marks to advertise territory ownership. Scent marks are the physical proof of the owner's ability to defend the scent marked area, as only a successful territory holder can ensure that the area is suffused with his fresh scent. Dominant territory owners seek out and attack other males that deposit competing scent marks in their territory and counter-mark by depositing their own urine nearby. Other males use these scent marks to recognise the territory owner and will usually flee if they meet the owner or may avoid entering a scent-marked area if the owner has defeated them in the recent past. This considerably reduces the need for aggressive defence by the owner. However, when a male does not defend his territory very effectively, other mice detect the presence of competitor scent marks and are themselves more likely to challenge the owner for dominance of the territory.

Because scent marks remain in the environment, they provide a continuous record of the outcome of competitive challenges between males. This is used by females to select high quality mates. Volatile pheromones in the urine of dominant territory owners are strongly attractive to females. Females prefer males with exclusively scent-marked territories or those that have successfully counter-marked challenges from other males. They avoid inbreeding by preferring mates that smell dissimilar from their parents. Subordinate males, in response to repeated defeat by a territory owner, reduce the concentration of volatile male pheromones in their urine that provoke male aggression. They also greatly reduce their scent marking to avoid competing with the territory owner, but must continue to deposit a small number of scent marks to be recognised and tolerated as a familiar resident. The cost of this is that they are forced to advertise their subordinate status through their scent marks and they are no longer attractive to females.



Pattern of scent marks on the cage floor (traced after 30min) when two males were first singly-housed and subsequently established a dominance relationship and were placed on either side of a mesh barrier to measure scent marking behaviour. The subordinate male suppressed scent marking and deposited urine in a large pool away from the dominant male. Traces were obtained by Dr Caroline Payne.

Two olfactory systems

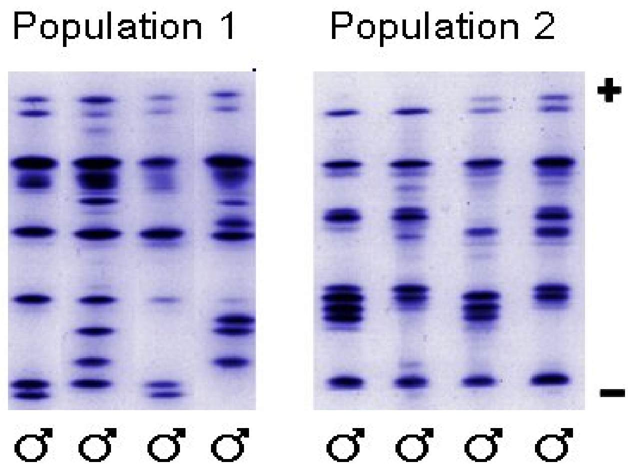
In addition to the main olfactory system that detects airborne volatiles, most mammals, reptiles and amphibia have a secondary olfactory system termed the vomeronasal system, although this is non-functional in humans. This secondary system is activated when animals make contact with a scent source and allows them to detect involatile as well as volatile scent components. The main olfactory system responds to a very broad range of odorants in the environment (e.g. food odours) while the vomeronasal system responds specifically to pheromones used for communication. In mouse urine, volatile pheromones are bound to involatile proteins that delay their release from scent marks. When mice detect an interesting scent in the air through their main olfactory system, their immediate response is to approach the scent source and sniff very closely. A vascular pumping mechanism is then activated that draws stimuli into paired vomeronasal organs which sit inside bony capsules just inside the nostrils. Contact investigation thus provides animals with much more information through the vomeronasal system than available from airborne odorants detected through the main olfactory system and many of the behavioural and priming effects of social odours depend on the ability to contact the scent. However, airborne volatiles are essential to detect the presence of a scent and to determine whether a scent is novel and requires further close contact investigation.

Table 1. Scent information in mouse urine

Genetic Identity FIXED	Current Status VARIABLE
• species	• social status
• sex	• reproductive status
• individual	• health
• kinship	• diet
• MHC type	• scent age (time since deposition)
• MUP type	• bacterial flora
	• metabolic variation

Individual recognition

Many genetic and non-genetic factors contribute to each animal's volatile scent profile (Table 1). To be useful, animals need to be able to distinguish between information about the scent owner's identity and its current status. The proteins and peptides in urine provide each individual with a genetically determined identity signature that indicates their species, sex and individual identity, in addition to other information such as the owner's kinship and MHC type, which remain fixed for life. The major urinary proteins (MUPs) and MHC peptides are so highly polymorphic among outbred wild mice that they provide each individual with an identity signature that is essentially unique. Mice can also learn to recognise the complex volatile profile of familiar individuals so that they can be recognised quickly from a distance from airborne scents. However, this volatile profile is less stable as it is also influenced by current status and environmental factors. Changes in the volatile scent profile induce further close contact investigation, which allows mice to confirm the identity of the scent owner through their involatile genetically-fixed identity signature.



The pattern of major urinary proteins (MUPs) expressed by different individual males captured from two populations. MUPs have been separated by isoelectric focusing according to their charge and stained with Coomassie Blue. Each individual has a different pattern. Gels run by Dr Caroline Payne.

The aggressive competition for breeding opportunities among males and their use of scents to advertise status and competitive ability have a number of consequences for aggression and welfare among laboratory mice. When males are confined together in cages, it is particularly important to avoid situations that provoke intolerant and injurious aggression through careful husbandry practices and to be aware of experimental treatments that might provoke an increase in aggression.

Aggression and scent communication among laboratory mice

The inbreeding of many strains of laboratory mice means that all individuals of the same sex and strain share the same genetically-determined scent signature. The effect of this is to generally lower levels of aggression between group-housed males of the same strain, as all males share the same signature as highly familiar group members. Problems of aggressive intolerance are thus usually greater among outbred males, although aggression within inbred strains can be increased by exposure to scents from a different strain or from the opposite sex. Some inbred strains are also genetically predisposed to high levels of aggression (e.g. SJL and BALB/c strains).

From both a management and welfare perspective, it is important to house animals in social groups whenever possible. Although female mice show little overt aggression (except towards intruders when pregnant or lactating), males of many laboratory strains can be highly aggressive under some circumstances. Apparently spontaneous outbreaks of intolerant aggression can occur even in relatively low aggression strains, resulting in serious wounding or even fatalities. Management practices can considerably reduce these problems by avoiding the stimuli that provoke aggression in all but completely non-aggressive strains.

- Adult males may be intolerant of unfamiliar individuals. This is particularly a problem among outbred mice where individuals each have different scent signatures. Males should be housed in their social groups before adulthood whenever possible so that they become familiar group members before attaining adult levels of aggression.
- Although mice of the same inbred strain share the same genetically-fixed identity signature and thus are more easily mixed during adulthood, an aggressive dominant male will still be intolerant of another aggressive male. If mice from different groups are mixed during adulthood, avoid housing more than one dominant (aggressive) male in the same group and observe carefully over the first few days.
- Males are more aggressive within their own soiled home cage as they are defending their scent-marked territory. Always introduce unfamiliar mice in clean cages, even when this is an inbred strain.
- Exposure to female odours strongly promotes aggression as males compete for opportunities to breed. Avoid housing females near male groups, particularly where substrate scents may fall into cages below. Avoid any exposure to female scents when males are removed from their cages (e.g. contact with contaminated hands, lab coats, soiled equipment). It may be impossible to regroup males used for breeding.
- Exposure to foreign male scents promotes aggression. Even exposure to a small amount of male urine when outside the home cage may increase aggression when mice are returned to the home group. This occurs even with male urine from the same inbred strain but is greatest when urine is from a different strain. Avoid contamination of the home cage with other male scents (e.g. cage bedding falling into the cage from above).
- Removal of soiled bedding odours from a scent-marked home cage may provoke increased aggression because subordinates' scents are removed while the dominant's scent marks

remain on the cage sides and grill. In aggressive outbred strains, avoid tipping out soiled substrate without washing the cage.

- Prolonged single housing will result in subsequent intolerance between males of more aggressive strains, as males begin to behave like exclusive territory owners. In less aggressive strains, pre-exposure to soiled bedding for several days prior to regrouping may help to familiarise mice to be housed together and reduce aggression.
- Changes to a mouse's body scent may prevent recognition and provoke attack on return to the home cage. Avoid contaminating mice with other scents during handling. If experimental procedures require application of substances that have a strong odour, familiarise animals by introducing the odour to the cage prior to the procedure. Treatments that have the potential to change the metabolism might change the animal's urinary scents. In aggressive strains, group-housed males should be observed closely to check that this does not stimulate an outbreak of intolerant aggression.
- Group housing of males from two or more inbred strains together can lead to greater aggression, even if males have only low levels of aggression when grouped within strain.
- Crossing two inbred strains beyond the F1 generation has the potential to create mice with different individual identity signatures, which is likely to lead to greater aggression particularly between unfamiliar mice (e.g. when backcrossing a transgenic onto a different genetic background).

Reproductive priming

In addition to the many influences of scent on behaviour, urinary scents from other mice in the local population prime the reproductive physiology and development of each individual according to the opportunities for reproduction. These effects can be stimulatory or inhibitory depending on the sex of both urine owner and recipient and their familiarity (Table 2).

Table 2. Reproductive priming effects of urine and soiled bedding in laboratory mice

	Urine donor		
	Novel adult male	***Non-breeding grouped females (>3)***	***Pregnant or lactating female***
Juvenile female	Accelerates puberty (Vandenburgh effect)	Delays puberty	Accelerates puberty
Adult female	Induces oestrus, shortens and synchronises oestrous cycle (Whitten effect)	Prolongs anoestrus or induces pseudopregnancy (Lee-Boot effect)	Prolongs oestrus
Pregnant female	Terminates early pregnancy, induces oestrus (Bruce effect)	No suppressive effects	
	Adult male	***Non-breeding grouped females (>3)***	***Non-crowded females (1-3)***
Juvenile male	Disrupts normal spermatogenesis, abnormal meiosis	Maturation of reproductive organs suppressed	Increased androgen production, faster maturation
Adult male	Suppresses sperm motility and attempts to mate in subordinates	Immunocompetence may be suppressed	Increased androgen production, increased sperm density

Cells shaded green are stimulatory effects, those shaded lilac are inhibitory. See Koyama (2004) for a detailed recent review.

Male scents on females: Male scents generally stimulate female reproductive physiology, bringing females into oestrus so that they are ready to mate. Young females may attain first oestrus up to 10 days earlier than normal when exposed to unfamiliar male scents (the familiar scent of their father has no such effects). If anoestrus adult females are allowed contact with male urine or soiled bedding, this initiates the oestrus cycle so that most females come into oestrus three days after exposure. This induction and synchrony of oestrus in adult females may be useful for timed matings in the laboratory or to ensure that females are at the same stage of the oestrus cycle in experiments. All of these stimulatory effects are induced by male pheromones acting through the vomeronasal system. Nasal contact with a male or his urine is therefore important to induce these effects and it is unlikely that airborne volatiles in the animal unit will have significant effects. However, contact may occur when soiled substrate is kicked out of cages and falls into those below, when animals of the opposite sex share soiled handling facilities, or by accidental transfer of their sticky urine via hands or equipment.

Importantly, contact with unfamiliar male urine can block pregnancy in a newly conceived female if she encounters the scent before the embryos implant into the wall of the uterus, returning her to oestrus so that she is available to mate again. However, continued contact with fresh scent from the male she has mated with helps to protect the pregnancy by maintaining prolactin levels so that embryos implant successfully. Urine from a male of the same inbred strain as the sire will not be detected as unfamiliar and will not block pregnancy as this shares the same individual identity cues as the sire.

Male scents on males: Less is known about reproductive priming effects on males, but scents from adult males generally have inhibitory effects on reproductive behaviour and sperm in other males, in addition to stimulating aggression. Urine from aggressive dominant males appears to be particularly potent in suppressing young and subordinate males.

Female scents on females: The effects of female scents depend on whether these come from reproductively active females or from non-reproductive females that live under crowded conditions. In crowded groups, non-reproductive females produce a pheromone (2,5-dimethylpyrazine) which affects other females by delaying puberty in young females and inhibiting oestrus cycling in adults. The greater the density of females and the more prolonged the exposure to this pheromone, the stronger the inhibitory effect. In wild populations, offspring survival is considerably reduced in overcrowded nest sites so this delays investment in offspring until females find more favourable conditions. In the laboratory, caged groups of more than three females generally produce this inhibitory pheromone but those in groups of 2-3 continue to cycle normally. Wild females prefer to nest communally with one or two other females as this increases offspring protection and survival. Correspondingly, urine from pregnant, lactating or singly housed oestrus females stimulates reproductive cycling in other females (see Table 2) and females are strongly attracted to nest with familiar companions. Single housing of females can lead to severe stereotypies and other abnormal behaviour which may have a significant impact on their responsiveness and variability in experiments.

Female scents on males: Exposure of males to female scents increases androgen production, stimulating faster maturation and increasing the aggressiveness of adult males. Bedding soiled by females also increases the number of sperm, particularly if the bedding comes from group-housed females. However, exposure to 2,5-dimethylpyrazine, which is produced at high levels by crowded non-reproductive females, has a negative influence, suppressing maturation of male reproductive organs and possibly suppressing their immunocompetence.

Controlling contact with priming pheromones is thus an important management consideration to reduce variability in physiology. The number of females housed in the same caged group is a particularly important consideration – the physiological status of those housed in small groups or singly will differ considerably from those housed in larger groups. Group size should therefore be considered carefully as part of the experimental design. The induction of oestrus by male scents may be a useful management tool not only in husbandry but also to synchronise female status in experiments.

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